Genetics

Cytochrome 2C19*17 Allelic Variant, Platelet Aggregation, Bleeding Events, and Stent Thrombosis in Clopidogrel-Treated Patients With Coronary Stent Placement

Dirk Sibbing, MD; Werner Koch, MD; Daniela Gebhard, MS; Tibor Schuster, MSc;
Siegmund Braun, MD; Julia Stegherr, MS; Tanja Morath, MS; Albert Schömig, MD;
Nicolas von Beckerath, MD; Adnan Kastrati, MD

Background—The cytochrome P450 (CYP) 2C19 isoenzyme plays an important role in clopidogrel metabolization. A recently explored *CYP2C19*17* allelic variant has been linked to increased transcriptional activity, resulting in extensive metabolization of *CYP2C19* substrates, which may lead to an enhanced platelet response to clopidogrel treatment. The aim of this study was to assess the impact of *CYP2C19*17* on ADP-induced platelet aggregation, the risk of bleeding, and stent thrombosis in clopidogrel-treated patients undergoing percutaneous coronary intervention.

Methods and Results—The study population included 1524 patients undergoing percutaneous coronary intervention after pretreatment with 600 mg clopidogrel. Genotypes were determined with a TaqMan assay. ADP-induced platelet aggregation was assessed on a Multiplate analyzer. The primary clinical safety end point was the 30-day incidence of bleeding defined according to Thrombolysis in Myocardial Infarction criteria, and the primary clinical efficacy end point was the 30-day incidence of stent thrombosis. For both heterozygous (*wt/*17; n=546) and homozygous (*17/*17; n=76) allele carriers, significantly lower ADP-induced platelet aggregation values were found compared with wild-type homozygotes (*wt/*wt; n=902; $P=0.039$ and $P=0.008$, respectively). *CYP2C19*17* allele carriage was significantly associated with an increased risk of bleeding; the highest risk was observed for *CYP2C19*17* homozygous patients ($P=0.01$, χ^2 test for trend). Multivariate analysis confirmed the independent association of *CYP2C19*17* allele carriage with platelet aggregation values ($P<0.001$) and the occurrence of bleeding ($P=0.006$). No significant influence of *CYP2C19*17* on the occurrence of stent thrombosis was found ($P=0.79$).

Conclusions—*CYP2C19*17* carrier status is significantly associated with enhanced response to clopidogrel and an increased risk of bleeding. (*Circulation*. 2010;121:512-518.)

Key Words: bleeding ∎ clopidogrel ∎ *CYP2C19* ∎ cytochrome ∎ stent thrombosis

In coronary artery disease patients undergoing percutaneous coronary intervention (PCI), aggressive antithrombotic and anticoagulant treatment regimens are routinely administered to reduce the risk of thrombotic complications. This risk reduction, however, comes with an increased risk of bleeding complications during and after the procedure.[1–3] Such periprocedural bleeding events are one of the most frequent complications after coronary stenting.[2,4–7] Recent findings suggest that bleeding occurring after a PCI procedure has an impact on 1-year mortality in patients that is similar to the occurrence of a post-PCI myocardial infarction.[2,7]

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Dual antiplatelet treatment with aspirin and clopidogrel is routinely administered to prevent thrombotic events after coronary stent placement; however, this therapy significantly contributes to the occurrence of bleeding events.[8]

Clopidogrel, an inactive prodrug, requires metabolization and activation by the hepatic cytochrome P450 (CYP) system to generate its active thiol metabolite, which targets and irreversibly inhibits the ADP P2Y12 receptor.[9] Hepatic metabolization of clopidogrel is achieved by a number of different hepatic CYP isoenzymes, including *CYP2C19*, 3A4/5, 1A2, 2B6, and 2C9. Evidence is accumulating that the polymorphically expressed isoenzyme *CYP2C19* constitutes a dominant part in this process.[10–16] A loss-of-function polymorphism in the *CYP2C19* gene, known as the

CYP2C19*2 allelic variant, has been associated with higher levels of ADP-induced platelet aggregation values in clopidogrel-treated patients and consequently a higher risk of major adverse cardiovascular events, including the occurrence of stent thrombosis (ST).[14–16]

In contrast to the numerous studies linking high postclopidogrel treatment platelet reactivity to an increased risk of ischemic events, including ST,[17–22] there is still a large gap in our understanding of the relation of an aggravated response to clopidogrel and bleeding events. A novel allelic variant, *CYP2C19*17*, has been discovered recently and results in an increased enzyme function of *CYP2C19* because of a mutation ($-808C>T$) in the 5′-flanking region of the gene that causes an increased transcription of *CYP2C19*.[23] Such increased transcriptional activity of *CYP2C19* may confer a rapid metabolization of *CYP2C19* substrates, which may lead to an enhanced response to antiplatelet treatment with clopidogrel. Although this may improve the prevention of thrombotic events, it also may increase the risk of bleeding. Thus, the aim of this study was to assess the impact of *CYP2C19*17* on ADP-induced platelet aggregation, the risk of bleeding events, and ST in clopidogrel-treated patients with coronary stent placement.

Methods

Patients

Between February 2007 and April 2008, patients with coronary artery disease and planned drug-eluting stent placement were enrolled in this study. Patients were consecutively recruited at the Deutsches Herzzentrum München (Munich, Germany) in the setting of a prospective trial including 1608 patients with platelet function testing on the Multiplate analyzer (Dynabyte, Munich, Germany).[17] For the present prespecified analysis, blood for DNA extraction and subsequent genotyping was available for 1524 patients (95%) of this cohort, which constitutes the study population for the present study. Sensitivity analysis confirmed that patients without DNA available (n=84) from the primary study population (n=1608) did not differ in terms of age, risk factors for coronary artery disease, platelet aggregation measurements, and clinical outcome (bleeding and ischemic events; $P>0.05$ for all of the variables investigated). The design of the primary trial has been described in detail.[17] All of the patients included in this study were pretreated with a loading dose of 600 mg clopidogrel before the procedure. The recommended pretreatment interval was $\geq$2 hours. Coronary interventions were performed according to current standard guidelines.[17] Intravenous anticoagulative treatment with unfractionated heparin was given in the majority of patients, and only some of the patients received bivalirudin. A small subset of the patients (<5%) received intravenous antiplatelet therapy with the glycoprotein IIb/IIIa inhibitor abciximab (bolus of 0.25 mg/kg of body weight, followed by an infusion of 0.125 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 12 hours) in addition to a reduced dose of heparin. In the time period after the procedure, patients were treated and discharged with a dual antiplatelet regimen of 75 mg clopidogrel (once per day) and 100 mg aspirin (twice per day). For this study, patients were considered eligible regardless of the clinical presentation (stable angina, unstable angina, ST-elevation myocardial infarction, and non–ST-elevation myocardial infarction) at the time of the procedure. Exclusion criteria were contraindications to aspirin or clopidogrel treatment and prior treatment with glycoprotein IIb/IIIa inhibitors during the 10 days before the PCI. The present study complies with the Declaration of Helsinki and was approved by the institutional ethics committee. All of the patients gave written informed consent for the intervention, platelet function testing, and genotype determination before study inclusion.

Blood Sampling and Genotyping

Blood for genomic DNA extraction and genotyping was taken from the arterial sheath of all of the patients directly before PCI. Genomic DNA was extracted from 200 μL blood with commercially available kits (NucleoSpin Blood Quick Pure, Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. Genotypes were determined with a TaqMan assay using an ABI Prism Sequence Detector 7000 (Applied Biosystems, Foster City, Calif) according to standard protocols. Primers 5′-GTTTGGAAGTTGTTTTGTT-TTGCTAAA-3′ and 5′-ACTGGGATTTGAGCTGAGGTCTT-3′ were used to amplify the sequence of the *CYP2C19* gene containing the single nucleotide polymorphism $-806C>T$ (rs12248560) in the 5′-flanking region of the gene. The sequence of the C-allele–specific probe was 5′-FAM-TTCTCAAAGCATCTCTGAT-3′, and the sequence of the T-allele–specific probe was 5′-VIC-TGTTCTC-AAAGTATCTCTGAT-3′. Genotypes were determined without knowledge of the patient's platelet aggregation values and clinical outcome. To control for correct sample handling, genotyping was repeated in 20% of the patients. Repeated genotyping revealed identical results.

Platelet Function Testing

For platelet function testing with multiple electrode platelet aggregometry, whole blood was obtained from the arterial sheath of all of the patients directly before PCI and was placed in 4.5-mL plastic tubes containing the anticoagulant lepirudin (25 μg/mL, Refludan, Dynabyte, Munich, Germany). ADP (6.4 μmol/L)-induced platelet aggregation was assessed with multiple electrode platelet aggregometry using a Multiplate analyzer. Details of this method have been reported previously.[17,24] Aggregation measured with multiple electrode platelet aggregometry is quantified as arbitrary units (AU) and area under the curve of arbitrary units (AU×minute). All of the material used for platelet function testing was obtained from the manufacturer. All of the measurements were obtained by laboratory personnel who were unaware of the results of genotyping and the clinical outcome of patients.

Study End Points and Definitions

For the analysis of the impact of the *CYP2C19*17* allelic variant on platelet aggregation after administration of clopidogrel, the ADP-induced platelet aggregation value was assessed from blood taken directly before the PCI. The primary clinical safety end point of the study was the 30-day incidence of combined major and minor bleeding events defined according to the Thrombolysis in Myocardial Infarction (TIMI) criteria (combined TIMI major and minor bleedings). The primary clinical efficacy end point of this study was the cumulative incidence of definite or probable ST during a 30-day follow-up period. Definite ST was defined according to the Academic Research Consortium criteria as the occurrence of an acute coronary syndrome with either angiographic or pathological confirmation of thrombosis.[25] Probable ST was defined as any unexplained death within 30 days or as target vessel myocardial infarction without angiographic confirmation of thrombosis or other identified culprit lesion. We further assessed the incidence of 30-day death and myocardial infarction. The diagnosis of myocardial infarction was made according to TIMI criteria and based on new abnormal Q-wave appearance in the ECG and/or an increase in the creatine kinase-MB value to $\geq$3 times the upper limit of normal. All of the events were adjudicated by an event adjudication committee blinded to the genotype and platelet function measurements of the patients.

Follow-Up

Patients stayed in the hospital for at least 2 days after study inclusion and after PCI. Patients were interviewed by telephone after 30 days ($\pm$7 days). Patients with cardiac symptoms were seen in the outpatient clinic for complete clinical, ECG, and laboratory checkup. Patient data were collected and entered into a computer database by specialized personnel, and all of the pertinent information from referring physicians, relatives, and hospital readmissions was entered. Source documentation was checked to ensure high-quality data.

Statistical Methods

Variables are presented as mean±SD, count (percentage), or median with interquartile range (IQR). Categorical variables were compared by use of the χ^2 test. The Kolmogorov-Smirnov test was used to check for normal distribution of continuous data. Normally distributed continuous data were compared between groups with 1-way ANOVA. Nonnormally distributed continuous data were compared between genotype groups by the Kruskal-Wallis test. Platelet function data obtained with multiple electrode platelet aggregometry were not normally distributed, are presented as median (IQR), were compared across all of the genotype groups with the Kruskal-Wallis test, and were compared between 2 groups with 2-sided unpaired Wilcoxon test.

We tested for a possible deviation of *CYP2C19*17* genotype distribution from Hardy-Weinberg equilibrium proportions using the Pearson goodness of fit χ^2 test. Differences between *CYP2C19*17* genotypes (wt/wt, wt/*17, *17/*17) with respect to clinical events were assessed by a χ^2 test for trend. Bootstrap sampling analysis was performed to achieve nonparametric 95% confidence intervals (CIs) for differences in medians between independent patient groups. A multiple logistic regression model was used to test for an independent association of *CYP2C19*17* allele carriage with TIMI bleeding events. Combined TIMI major and minor bleedings were defined as the dependent variable. Independent variables were *CYP2C19*17* carrier status and variables with a reported significant influence on the occurrence of bleeding events after PCI across different studies,[1,4,5] namely age, sex (female versus male), and body mass index. In addition, adjustment was also made for the use of proton pump inhibitors, use of abciximab during the PCI procedure, renal function (serum creatinine), and the clopidogrel loading interval. The odds ratio (OR) and the corresponding 95% CI were calculated for each variable included in the multivariate model. Furthermore, a multivariable linear regression model was used to test for an independent association of *CYP2C19*17* allele carriage with ADP-induced platelet aggregation measurements (dependent variable). Independent variables were *CYP2C19*17* carrier status and all of the variables that were included in the primary multivariable logistic regression model with combined TIMI major and minor bleeding as the dependent variable. Use of abciximab was excluded here because platelet function testing was done in all of the patients before the administration of PCI-related abciximab treatment. All of the analyses were performed with the S-PLUS software package (Insightful Corp, Seattle, Wash). For all of the statistical analyses, a value of $P<0.05$ was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Population and *CYP2C19*17* Genotyping

Baseline characteristics of the study population according to the *CYP2C19*17* genotypes are shown in Table 1. Variables were well balanced between the 4 genotype groups. Of the 1524 patients included in this study, 902 (59%) were wild-type homozygous for the *17 allelic variant (wt/wt), 546 (36%) were *CYP2C19*17* heterozygotes (wt/*17), and 76 (5%) were homozygous (*17/*17) for the mutant *CYP2C19*17* allelic variant. Consequently, 622 patients (41%) were carriers of at least one *17 allele. This genotype distribution results in the following allele frequencies: 77.1% for the *CYP2C19* wild-type allele versus 22.9% for the *CYP2C19*17* mutant allelic variant. For genotype distribution, no significant deviation from Hardy-Weinberg equilibrium was observed ($P=0.77$).

**Table 1. Baseline Characteristics of the Study Population**

Variable	CYP2C19 wt/wt (n=902)	CYP2C19 wt/*17 (n=546)	CYP2C19 *17/*17 (n=76)	P
Age, y	67.5±10.7	67.2±10.4	67.4±9.6	0.82
Sex, female, n (%)	194 (21.5)	130 (23.8)	20 (26.3)	0.43
Body mass index, kg/m^2	27.4±4.4	27.7±4.0	27.9±5.1	0.21
Ejection fraction, %	54.6±11.3	54.9±11.0	55.2±10.1	0.98
Serum creatinine, mg/dL	1.03±0.5	1.02±0.4	1.02±0.6	0.39
Diabetes mellitus, n (%)	239 (26.5)	170 (31.1)	21 (27.6)	0.16
Active smoker, n (%)	131 (14.5)	61 (11.2)	15 (19.7)	0.05
Arterial hypertension, n (%)	824 (91.4)	499 (91.4)	69 (90.8)	0.98
Hypercholesterolemia, n (%)	633 (70.2)	380 (69.6)	55 (72.4)	0.88
Family history of CAD, n (%)	376 (41.7)	227 (41.6)	39 (51.3)	0.25
Previous MI, n (%)	286 (31.7)	179 (32.8)	21 (27.6)	0.65
Previous bypass surgery, n (%)	129 (14.3)	84 (15.4)	10 (13.2)	0.79
Multivessel disease, n (%)	766 (84.9)	464 (84.9)	62 (81.6)	0.72
Non-STEMI/STEMI, n (%)	106 (11.8)	59 (10.8)	4 (5.3)	0.22
Platelet count, ×10^3/μL	217±63	218±65	219±54	0.71
Clopidogrel loading interval, h	3.5 (2.0–13.5)	3.5 (2.0–15.0)	5.3 (2.4–16.2)	0.14
Use of abciximab, n (%)	22 (2.4)	11 (2.0)	1 (1.3)	0.75
Medication at admission, n (%)				
Aspirin	680 (75.4)	418 (76.6)	57 (75.0)	0.87
Thienopyridine	391 (43.3)	229 (41.9)	24 (31.6)	0.13
β-blocker	650 (72.1)	418 (76.6)	62 (81.6)	0.05
ACE inhibitor	520 (57.6)	302 (55.3)	42 (55.3)	0.66
AT1 inhibitor	167 (18.5)	115 (21.1)	15 (19.7)	0.49
Coumarine derivatives	99 (11.0)	59 (10.8)	6 (7.9)	0.71
Statins	620 (68.7)	391 (71.6)	51 (67.1)	0.45
Proton pump inhibitors	170 (18.9)	96 (17.6)	10 (13.2)	0.43

CAD indicates coronary artery disease; MI, myocardial infarction; STEMI, ST-elevation MI; ACE, angiotensin-converting enzyme; and AT1, angiotensin 1. Data presented are mean±SD when appropriate. Clopidogrel loading interval denotes the time from clopidogrel loading to platelet function testing and is shown as median (IQR).

*CYP2C19*17* and Platelet Aggregation

The median value of ADP-induced platelet aggregation in the study population was 226 AU×min (IQR, 141 to 364 AU×min). The median ADP-induced platelet aggregation values across *CYP2C19*17* genotypes were as follows: 238 AU×min (IQR, 146 to 388 AU×min) for wt/wt patients, 215 AU×min (IQR, 140 to 342 AU×min) for wt/*17 patients,
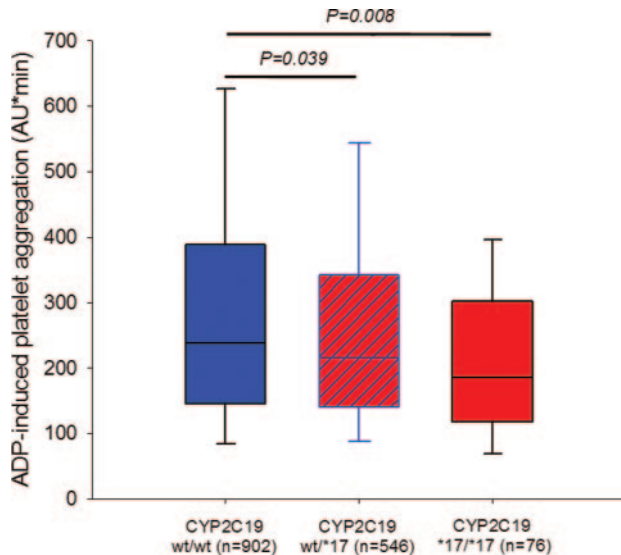
Figure 1. *CYP2C19*17* genotypes and platelet aggregation. ADP-induced platelet aggregation (AU×minute) in relation to *CYP2C19* genotypes (wt/wt, wt/*17, *17/*17). Platelet aggregation values were compared across all genotype groups with the Kruskal-Wallis test (*P*=0.007) and between groups with unpaired 2-sided Wilcoxon test.

and 186 AU×min (IQR, 119 to 301 AU×min) for *17/*17 patients. ADP-induced platelet aggregation was significantly different between the 3 genotype groups (*P*=0.007). As demonstrated in Figure 1, the lowest ADP-induced platelet aggregation values were observed for patients carrying 2 of the mutant *CYP2C19* alleles (*17/*17). In the 622 patients who were carriers of at least 1 *CYP2C19*17* allele (wt/*17 or *17/*17), ADP-induced platelet aggregation was lower compared with wild-type homozygous (wt/wt; n=902) patients (213 AU×min [IQR, 136 to 329 AU×min] versus 238 AU×min [IQR, 146 to 388 AU×min], respectively; *P*=0.009). Bootstrap sampling analysis (1000 bootstrap samples from original data) revealed a mean difference in medians of AU×minute for carriers of at least 1 *CYP2C19*17* allele versus noncarriers of 24 AU×min (95% CI, 12 to 36). Results of a multivariable linear regression model (Table 2) demonstrated that *CYP2C19*17* allele carriage was independently associated with ADP-induced platelet aggregation measurements.

Table 2.   Results of a Multivariable Linear Regression Model

Variable	β Coefficient		
	Value	SE	P
CYP2C19*17 allele carriage	−32.3	9.5	0.0006
Age	0.2	0.5	0.75
Sex	34.4	13.9	0.01
Body mass index	4.5	1.3	0.0006
Serum creatinine	13.3	13.1	0.31
Use of proton pump inhibitors	26.2	14.6	0.07
Clopidogrel loading interval	−1.9	0.4	<0.0001

Results of the multivariable linear regression model are shown for ADP-induced platelet aggregation as the dependent variable. Independent variables, including *CYP2C19*17* allele carriage, are shown. Unadjusted β coefficient for *CYP2C19*17* allele carriage was −32.0 (SE=9.5).
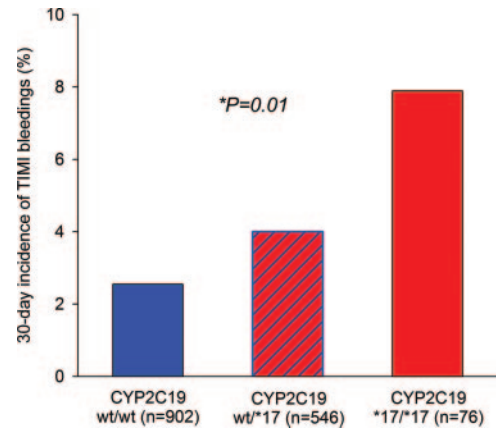
Figure 2. *CYP2C19*17* genotypes and incidence of TIMI bleedings. Thirty-day incidence of TIMI major or minor bleedings in relation to *CYP2C19*17* genotypes (wt/wt, wt/*17, *17/*17). *Calculated with χ^2 test for trend.

*CYP2C19*17* and Bleeding Events

The primary safety end point (combined TIMI major and minor bleedings) within 30 days occurred in 51 patients (3.3%) of the study population. Twelve TIMI major bleedings (0.8%) and 39 TIMI minor bleedings (2.5%) were observed. Forty-five (88%) of the bleeding events observed occurred during the first 24 hours after the procedure. Forty-nine bleedings (96%) were in-hospital bleeding events. According to *CYP2C19*17* genotype, the risk of bleeding was higher in both heterozygous and homozygous carriers of the *CYP2C19*17* allele (wt/*17 and *17/*17 versus wt/wt: OR, 1.80; 95% CI, 1.03 to 3.14; see Figure 2). The risk of bleeding was highest in patients carrying 2 of the mutant *CYP2C19*17* alleles (*17/*17 genotype; χ^2 test for trend, *P*=0.01; wt/wt versus *17/*17: OR, 3.27; 95% CI, 1.33 to 8.10). Of the 51 bleeding events observed, 28 events (55%) occurred in patients with homozygous or heterozygous *CYP2C19*17* allele carriage. For TIMI major bleeding alone, the incidence according to *CYP2C19*17* genotype was as follows: 5 (0.6%) in wt/wt patients, 6 (1.1%) in wt/*17 patients, and 1 (1.3%) in *17/*17 patients (χ^2 test for trend, *P*=0.22; wt/*17 and *17/*17 versus wt/wt: OR, 2.04; 95% CI, 0.68 to 6.12; wt/wt versus *17/*17: OR, 2.39; 95% CI, 0.95 to 2.10). Among TIMI major bleedings, 2 fatal intracranial bleedings occurred. Both patients were carriers of the *CYP2C19*17* allele (patient 1: genotype, wt/*17; platelet aggregation, 168 AU×min; patient 2: genotype, *17/*17; platelet aggregation, 152 AU×min). For TIMI minor bleeding alone, the incidence according to *CYP2C19*17* genotype was as follows: 18 (2.0%) in wt/wt patients, 16 (2.9%) in wt/*17 patients, and 5 (6.6%) in *17/*17 patients (χ^2 test for trend, *P*=0.025; wt/*17 and *17/*17 versus wt/wt: OR, 1.72; 95% CI, 0.92 to 3.22; wt/wt versus *17/*17: OR, 3.46; 95% CI, 1.30 to 9.27). To test for an independent association of *CYP2C19*17* carrier status and TIMI bleeding events, a multiple logistic regression model was used that included *CYP2C19*17* carrier status and possible confounding variables. Results of this multiple logistic regression model (Table 3) demonstrated that carriage of the *CYP2C19*17* allele was an independent predictor of 30-day TIMI bleedings (OR, 1.85; 95% CI, 1.19

Table 3.   Results of a Multivariable Logistic Regression Model

Variable	OR (95% CI)	P
*CYP2C19*17* allele carriage	1.85 (1.19–2.86)	0.006
Age (per 10-y increment)	1.57 (1.13–2.17)	0.006
Sex	1.31 (0.68–2.54)	0.42
Body mass index (per 5-kg/m^2 increment)	0.87 (0.61–1.25)	0.46
Serum creatinine (per 0.1-mg/dL increment)	0.97 (0.88–1.06)	0.49
Use of proton pump inhibitors	1.21 (0.60–2.45)	0.59
Use of abciximab	5.12 (1.83–14.31)	0.002
Clopidogrel loading interval (per 1-h increment)	1.00 (0.99–1.02)	0.70

Results of the multivariable logistic regression model are shown for combined TIMI major and minor bleeding as the dependent variable. Independent variables, including *CYP2C19*17* allele carriage, are shown. The clopidogrel loading interval denotes the time from clopidogrel loading (in hours) to platelet function testing. Unadjusted OR for *CYP2C19*17* allele carriage, 1.80; 95% CI, 1.03–3.14.

to 2.86 for *CYP2C19*17* allele carriage; OR, 3.41; 95% CI, 1.42 to 8.17 for homozygous *CYP2C19*17* allele carriage versus no *17 allele carriage; $P=0.006$).

*CYP2C19*17* and Ischemic Events

The primary efficacy end point (combined definite or probable ST) within 30 days occurred in 14 patients (3.3%) of the study population. Ten definite STs and 4 probable STs according to Academic Research Consortium criteria were observed. The incidence of 30-day ST according to *CYP2C19*17* genotype was similar between the genotype groups: 8 (0.9%) in wt/wt patients, 5 (0.9%) in wt/*17 patients, and 1 (1.3%) in *17/*17 patients (χ^2 test for trend, $P=0.79$; wt/*17 and *17/*17 versus wt/wt: OR, 1.09; 95% CI, 0.39 to 3.02). Thirty-day mortality rates according to *CYP2C19*17* genotype were as follows: 5 (0.6%) in wt/wt patients, 2 (0.4%) in wt/*17 patients, and 1 (1.3%) in *17/*17 patients (χ^2 test for trend, $P=0.84$; wt/*17 and *17/*17 versus wt/wt: OR, 0.87; 95% CI, 0.23 to 3.31). No significant differences were observed for the incidence of 30-day myocardial infarction: 29 (3.2%) in wt/wt patients, 17 (3.1%) in wt/*17 patients, and 4 (5.3%) in *17/*17 patients (χ^2 test for trend, $P=0.61$; wt/*17 and *17/*17 versus wt/wt: OR, 1.05; 95% CI, 0.59 to 1.85). In addition, no significant differences were observed for the incidence of a combined 30-day ischemic end point (death/myocardial infarction/urgent target vessel revascularization): 34 (3.7%) in wt/wt patients, 18 (3.2%) in wt/*17 patients, and 4 (5.3%) in *17/*17 patients (χ^2 test for trend, $P=0.94$; wt/*17 and *17/17 versus wt/wt: OR, 0.94; 95% CI, 0.55 to 1.61).

Discussion

To the best of our knowledge, this is the first study to report on the impact of the *CYP2C19*17* allelic variant on both platelet response to clopidogrel and the occurrence of bleeding events or ST in a large population of patients undergoing coronary stent placement. Major findings of our study are that (1) the *CYP2C19*17* allelic variant has a significant impact on ADP-induced platelet aggregation in clopidogrel-treated patients, resulting in an enhanced response to clopidogrel in the presence of the *17 allele; (2) the presence of the *17

allele confers to an increased risk of bleeding events; and (3) the *17 allele does not have a protective effect on the occurrence of ST or other ischemic events.

Results of our study are strengthened by the fact that we observed a pronounced gene-dose effect for the mutant *CYP2C19*17* allele on both platelet function and the occurrence of bleeding events, with the lowest ADP-induced platelet aggregation values and correspondingly the highest risk of bleeding events observed in *17/*17 homozygous patients. Specifically, for the group of patients homozygous for the *CYP2C19*17* allelic variant, an ≈4-fold increase in the occurrence of a bleeding event was observed. Applying a multivariate analysis to our data, we were able to demonstrate that a highly significant influence of the *17 allele on the incidence of bleeding events remained even after adjustment for possibly confounding variables, including known predictors of bleeding after coronary stent placement. Despite the significant impact of the *17 allele on bleeding events, we did not observe an influence of *CYP2C19*17* allele carriage on ST, which is in line with the results reported by Simon et al,[16] who found no influence of *CYP2C19*17* on the risk of death resulting from any cause, nonfatal stroke, or myocardial infarction during a 1-year follow-up period. Two reasons may account for this finding. First, the total number of observed STs (n=14) was lower than the event rate of TIMI major or minor bleedings (n=51), making it difficult to find significant differences across *CYP2C19*17* genotypes, and it cannot be excluded that an even larger subset of patients with more events would experience a significant influence in terms of a protective effect of the *17 allele on the incidence of ST. Second, the *CYP2C19*17* allelic variant shifts ADP-induced platelet aggregation to lower values, which may not necessarily have a protective effect on the occurrence of ischemic events such as ST, which increase substantially above certain cutoff values.[17]

Recent mechanistic investigations have demonstrated that the transcriptional activity of the *CYP2C19* gene is significantly upregulated in the presence of the *17 allele, which is defined by a mutation at position 806 (−806C>T) in the 5′-flanking region of the gene.[23] The *17 allele can specifically bind nuclear proteins to the 5′-flanking region of the gene, which leads to increased gene transcription and expression. Consequently, the presence of the *17 allele cosegregates with an ultrarapid metabolism of *CYP2C19* substrates. This has been demonstrated consistently in a number of pharmacological studies.[23,26–28]

Data are limited investigating the influence of *CYP2C19*17* on the metabolism of clopidogrel. In a smaller subset of patients (n=237), Geisler et al[15] assessed the impact of the *17 allele in clopidogrel-treated patients who underwent PCI. A trend was observed toward lower levels of residual ADP-induced platelet aggregation in carriers of the *17 allele. Because of the relatively small number of patients, however, the differences did not reach statistical significance (OR, 0.62; 95% CI, 0.34 to 1.14; $P=0.14$). From the highly significant results obtained in the present larger study population, it must be assumed that generation of the active thiol metabolite is significantly increased in *CYP2C19*17* carriers. An association of thiol metabolite levels and ADP-induced

platelet aggregation values achieved has been demonstrated in a number of studies[29–31]: High active thiol metabolite levels in turn lead to low ADP-induced platelet aggregation values, which are likely to provide the basis for the significantly increased risk of bleeding complications in the early period after the procedure, which was observed in this group of patients.

A number of clinical studies have demonstrated the significant impact of periprocedural bleeding events on early[3–5,7] and long-term mortality.[2,4,5,7] For the primary end point of TIMI bleedings, which was chosen in our study, a huge impact on 1-year mortality has been demonstrated.[2] Recent studies were able to define predictors of periprocedural bleeding events such as age, sex, or body mass index[1,2,4,5]; however, a large gap in our knowledge exists about how bleeding events may be related to platelet response to clopidogrel treatment and certain genetic factors with an influence on clopidogrel metabolism. This circumstance and the results from clinical studies showing the poor prognosis of patients with recent bleeding events[2,7] accentuate the need to define a specific subgroup of patients in whom the risk of developing major or minor bleeding events is substantially increased. The results of the present study identify those patients at high risk for bleeding complications in the setting of clopidogrel treatment and coronary stent placement. By establishing a genetic risk factor in the form of the *CYP2C19*17* allele, which is closely linked to the metabolism of clopidogrel, we can better characterize these patients before the administration of antiplatelet treatment regimens. It could be speculated that an intensified antiplatelet treatment with clopidogrel could be derogatory, especially to this group of patients.

Results of the present study and other studies indicate the need to further define a therapeutic window for oral antiplatelet treatment with clopidogrel or newer agents such as prasugrel. Not only does the risk for ischemic events substantially increase above a certain threshold value of ADP-induced platelet aggregation[17–20] but the risk for bleeding events may also increase in patients with low ADP-induced platelet aggregation values caused by the *CYP2C19*17* allelic variant. Genotyping for relevant gene polymorphisms[10,23] in the hepatic CYP system may help to individualize and optimize oral antiplatelet treatment. Specifically designed studies are needed that directly link a beneficial outcome of patients undergoing coronary stent placement with antiplatelet treatment regimens based on genetic or platelet function testing. Results of our study and other recently published large-scale trials[12,13,16] may provide the rationale for such studies. For the individual patient undergoing coronary stent placement, the information provided by genetic and platelet function testing may be complementary in improving patients' outcomes.

The present study has limitations that merit mention. We assessed the impact of only 1 single genetic and functionally relevant variant on ADP-induced platelet aggregation and patient clinical outcome. The interaction of a number of genetic variants and their combined impact on clinical outcome and platelet aggregation measures were not studied. A limitation of the study is related to the low number of adverse events observed despite the large number of patients included in this study. This reflects the increased safety associated with PCI but also underscores the need for further studies to corroborate the present results. A further limitation of the present study is that this analysis was a post hoc analysis of a study population that stems from a prospective trial; therefore, it is subject to the limitations inherent to all such analyses.

Conclusions

CYP2C19*17 carrier status is significantly associated with an enhanced response to clopidogrel treatment and an increased risk of bleeding.

Sources of Funding

Disclosures

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CLINICAL PERSPECTIVE

Responsiveness to clopidogrel treatment, as assessed in vitro by the measurement of ADP-induced platelet aggregation, is characterized by large interindividual variability. Such variability is to a significant degree related to certain genetic risk factors that significantly affect clopidogrel bioactivation. Although a number of studies have convincingly demonstrated the association of the *CYP2C19*2* loss-of-function polymorphism with both high ADP-induced platelet aggregation values and a remarkably increased risk for ischemic events, including stent thrombosis, a large gap in knowledge exists in our understanding of how other genetic variants may trigger bleeding events caused by enhanced clopidogrel bioactivation. The present study of 1524 clopidogrel-treated patients undergoing coronary stenting demonstrates that the *CYP2C19*17* allelic variant has a significant impact on ADP-induced platelet aggregation in clopidogrel-treated patients, resulting in an enhanced response to clopidogrel in the presence of the *17 allele. This study also shows that the presence of the *17 allele confers an increased risk of bleeding events in carriers of the allele. Therefore, knowledge of the *CYP2C19*17* genotype status, in addition to a panel of other established risk factors, can be clinically useful in better predicting bleeding complications in clopidogrel-treated patients undergoing coronary stent placement.

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Dirk Sibbing, Werner Koch, Daniela Gebhard, Tibor Schuster, Siegmund Braun, Julia Stegherr, Tanja Morath, Albert Schömig, Nicolas von Beckerath and Adnan Kastrati

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